ELSEWHERE IN BIOLOGY

A selection of interesting papers and reviews published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*.

Chosen and summarized by the staff of *Chemistry & Biology*.

Chemistry & Biology February 1996, **3**:139–141

► Cell–cell interactions: **Taking cues from the neighbors**
Detlef Weigel and Peter Doerner (1996). *Curr. Biol.* **6**, 10–12.

The rigidity of plant cell walls constrains the interactions between neighboring cells, and suggests that plants may not be the ideal system to observe signaling between cells. Recent studies are demonstrating, however, that plant cell identity shows surprising plasticity, and is maintained by complex cell–cell signaling. Cells that have been killed with a laser can be replaced by cells that are dividing nearby, which change fate in response to their new position. This fate change occurs between lineages that diverged as far back as the first zygotic division. Other ablation experiments have identified certain cells required for asymmetric division events, and a temperature-sensitive transposon has been used to mark small lineages of cells, demonstrating that lineage restriction between flower whorls occurs relatively late in flower development. Which molecules mediate these inductive interactions? One candidate is the product of the *SUPERMAN* (*SUP*) gene, a transcription factor necessary for restricting the expression of organ-identity genes such as *APETALA3* (*AP3*) and *PISTILLATA* (*PI*). Surprisingly, *AP3* and *PI*, which promote stamen development, are positive regulators of *SUP*. In the small region in which it is expressed, *SUP* may prevent the proliferation of *AP3*/*PI*-expressing cells, thus delimiting the whorl boundary. Diffusible mediators of these signals remain to be identified, and some evidence implicates carbohydrates rather than proteins.
1 January 1996, Dispatch, *Current Biology*

► Macromolecular complexes: **How RNA and protein get together**
Sarah A Woodson (1996). *Curr. Biol.* **6**, 23–25.

We now know that many ribonucleoprotein structures (such as ribosomes and spliceosomes) are essentially RNA machines, in which the proteins have auxiliary functions. The question remains as to how RNA–protein interactions contribute to the activity of the complex. One clue comes from the cytochrome b pre-mRNA processing protein 2 (CBP2), which is required for splicing of an intron in the yeast cytochrome b pre-mRNA at physiological concentrations of Mg^{2+}. Tertiary structure and base pairing of the self-splicing RNA were probed by hydroxyl radical cleavage and methylation, respectively. In the absence of CBP2, the core of the intron can fold in a Mg^{2+}-dependent manner, but the 5' domain that contains the 5' splice-site helix remains accessible. Addition of CBP2 further protects the core and the 5' domain. CBP2 binds the fully-folded intron, however, and therefore does not accelerate the rate of folding.
1 January 1996, Dispatch, *Current Biology*

► Neural Development: **Identical twins separated at birth?**
Christopher A Walsh (1996). *Curr. Biol.* **6**, 26–28.

Understanding the specification of four incipient neuroblasts in *Drosophila* is a far more reasonable task than understanding the development of the mammalian cerebral cortex, and yet similar mechanisms may underlie both events. In both systems activation of Notch (by binding its ligand Delta) activates *Enhancer of Split* (or its homolog *HES-1*), which inhibits neurogenesis. A new system for directly observing cerebral cortex divisions in real time has demonstrated alterations in the cleavage planes of the cells as neurogenesis proceeds. Before neurogenesis begins, 90 % of cleavages are vertical and generate two indistinguishable daughter cells. Later in development, up to 50 % of divisions are horizontal, generating one apical cell that stays near the ventricular surface, and one basal cell that migrates away and which may represent a post-mitotic neuron. Furthermore, all or most of the Notch1 protein is retained in the basal daughter cell. The localization of Notch to the presumptive neuron is paradoxical given the previously defined function of Notch in inhibiting neurogenesis, and may be a clue that Notch is multifunctional, or that its primary function is to temporarily maintain cells in an undifferentiated state.
1 January 1996, Dispatch, *Current Biology*

► T-cell costimulation: **T cells themselves call the shots**
Trevor Owens (1996). *Curr. Biol.* **6**, 32–35.

Signals transduced through T-cell or B-cell antigen receptors are augmented by CD40 and its ligand, CD40L, in a process known as costimulation. CD40 is expressed by B cells and various antigen presenting cells; CD40L is expressed transiently by $CD4^+$ T cells following their activation. The binding of CD40L to CD40 has been implicated in triggering many responses in B cells. Recent evidence suggests that binding of CD40L to its receptor regulates T cells themselves, by the induction of a novel costimulator on B cells (and perhaps, by analogy, on antigen presenting cells) that feeds back to activate the T cells. The loss of costimulation on antigen-presenting cells could explain the failure of $CD40L^-$ T cells to clonally expand after recognition of a foreign antigen for the first time. Failure of costimulation may also be the mechanism underlying the escape of potentially self-reactive T cells from the thymus in $CD40L^-$ mice. It is not a complete surprise to find a new costimulatory activity, as mice deleted for other costimulatory molecules retain some T-cell responses. It seems that costimulation involves multiple players in parallel pathways and amplification loops.
1 January 1996, Dispatch, *Current Biology*

► Immunology: **Why the baby isn't thrown out...**
Elizabeth Simpson (1996). *Curr. Biol.* **6**, 43–44.

How does the fetus, expressing paternal as well as maternal antigens, survive in the potentially hostile environment of the uterus? A recent study addressing this question uses female mice expressing a transgenic T-cell receptor that recognizes only a specific paternal antigen, so that changes in this population of cells can be readily followed. By mid-gestation, expression of the transgenic T-cell receptors was dramatically downregulated, a result similar to that seen in double transgenic mice expressing both the receptor and the corresponding antigen. The effects are reversed after the pregnancy, by a mechanism that is not dependent on newly emerged T cells. The suppression of the T-cell response is not due to antibodies preventing recognition of the the paternal antigens, as it still occurs in *Scid* mice, which do not produce any antibodies. Tolerance to fetal antigens probably

involves antigens that are developmentally regulated, and which may also be expressed in autoimmune diseases. Understanding how the fetus survives may therefore aid in the manipulation of harmful immune responses.
1 January 1996, Dispatch, *Current Biology*

► **Apoptosis and change of competence limit the size of the vulva equivalence group in *Pristionchus pacificus*: a genetic analysis**
Ralf J Sommer and Paul W Sternberg (1996). *Curr. Biol.* **6**, 52–59.

Different organisms are diverse in form, yet there is widespread conservation of developmental mechanisms. Can simple changes at the molecular level give rise to this diversity? Vulval development in nematodes is a well defined system suited to the study of this problem. In *Caenorhabditis elegans*, six of twelve ventral epidermal cells are competent to adopt vulval cell fates in response to an inductive signal and thus form the 'vulval equivalence group'. In *Pristionchus pacificus*, another species in the family Rhabditidae, the vulval equivalence group is limited by apoptosis and a decreased responsiveness to inductive signals (competence). Apoptosis is seen in the ventral epidermal cells of *C. elegans* only in the presence of gain-of-function mutations in the genes *lin-24* or *lin-33*. Close relatives of the ventral epidermal cells apoptose in wild-type worms, however, leaving open the possibility that the apoptosis in *Pristionchus* may occur by alteration of this pre-existing machinery. A *ped-5* mutation leads to survival of two anterior cells that die in wild-type *Pristionchus*, and these cells are now responsive to vulval inducing signals. This expansion could be explained by a loss of function in a homeotic gene that normally overrides the homeobox gene *lin-39*. Thus, alterations in the homeotic gene control of anteroposterior pattering may be involved in creating these evolutionary differences.
1 January 1996, Research Paper, *Current Biology*

► **Two GTPases, Cdc42 and Rac, bind directly to a protein implicated in the immunodeficiency disorder Wiskott–Aldrich syndrome**
Pontus Aspenström, Uno Lindberg and Alan Hall (1996). *Curr. Biol.* **6**, 70–75.

The Rho family of small GTPases is important in controlling cell shape and motility. Two of these proteins, Cdc42 and Rac, have been previously shown to induce the formation of filopodia and lamellipodia, respectively, at the cell periphery. Here, the yeast two-hybrid system is used to screen for proteins that interact with Cdc42, and WASP is identified. This protein has been implicated in the immunodeficiency disorder Wiskott–Aldrich Syndrome (WAS); recombinant WASP binds tightly to Cdc42 and weakly to Rac but not at all to Rho. Binding is dependent on Cdc42 or Rac being in the GTP-bound conformation, but WASP binding does not increase nucleotide exchange or the GTPase activity of Cdc42. WASP-deficient B and T lymphocytes have distorted and reduced numbers of cell-surface microvilli and have defects in transmembrane signaling. This study suggests that the phenotype may be due, at least in part, to a defect in the signal transduction pathways regulated by Cdc42/Rac.
1 January 1996, Research Paper, *Current Biology*

► **Inhibition of pRb phosphorylation and cell-cycle progression by a 20-residue peptide derived from p16$^{CDKN2/INK4A}$**
Robin Fåhraeus, Jesús M Paramio, Kathryn L Ball, Sonia Laín and David P Lane (1996). *Curr. Biol.* **6**, 84–91.

The gene encoding p16 is deleted in a large number of human cancers. The protein interacts directly with cdk4 and cdk6, and it inhibits phosphorylation of pRb, blocking the entry of cells into S phase. To identify the region of p16 responsible for interaction with cdk4/cdk6, a series of overlapping 20-residue peptides were synthesized. A peptide corresponding to amino acids 84–103 is shown to bind to cdk4 and cdk6 and to inhibit phosphorylation of pRb in cell extracts. The peptide also inhibits phosphorylation of pRb and entry of cells into S phase when coupled to a carrier peptide and applied to human keratinocytes in culture. Because the function of p16 can be mimicked by a short peptide, it is an attractive starting point for future peptidomimetic drug design aimed at cell-cycle inhibition and anticancer treatment.
1 January 1996, Research Paper, *Current Biology*

► **Adding backbone to protein folding: why proteins are polypeptides**
Barry Honig and Fred E Cohen (1996). *Folding & Design* **1**, R17–R20.

One extreme model of protein folding considers only the side-chains of polypeptides as important, with the backbone acting as inert carrier. This model fails to account for the limiting cases of polymers of all hydrophobic or all polar residues. Here it is argued that polypeptides are 'special' (in that they, unlike ordinary polymers, form unique three-dimensional structures) primarily because of their backbones. The removal of polar NH and CO groups from water involves a significant energetic penalty that must be compensated for largely by the formation of intramolecular hydrogen bonds. These hydrogen bonds give rise to periodically ordered conformations such as α-helices, and provide a strong structural constraint which excludes many of the conformations that would be predicted to be stable by sidechain-only models. The amino-acid sequence can then choose among the limited set of secondary structure possibilities available to the polypeptide backbone.
February 1996, Review, *Folding & Design*

► **Scrapie prions: a three-dimensional model of an infectious fragment**
Ziwei Huang, Stanley B Prusiner and Fred E Cohen (1996). *Folding & Design* **1**, 13–19.

A proposed mechanism for the infectivity of the prion protein suggests that the cellular (PrP^C) and scrapie (PrP^{Sc}) forms of the protein are stable, monomeric, conformational isomers. The α-helical PrP^C is proposed to convert to PrP^{Sc}, containing ~45% β-sheet. Here, a plausible model for the three-dimensional structure of a fragment of PrP^{Sc} (residues 108–218) is presented. The structure is a four-stranded β-sheet covered on one face by two α-helices, and residues implicated in the inability of prions to infect across species are clustered on the solvent accessible surface of the β-sheet. The authors propose that PrP^C is a kinetically trapped intermediate in PrP folding.
8 December 1995*, Research Article, *Folding & Design*

► **Stabilization of proteins by rational design of α-helix stability using helix/coil transition theory**
Virtudes Villegas, Ana Rosa Viguera, F Xavier Avilés and Luis Serrano (1996). *Folding & Design* **1**, 29–34.

As the stability of a given protein varies in different organisms, it is likely that most proteins have sub-optimal stabilities. Here a helix/coil transition algorithm is used to design mutations affecting solvent-exposed residues that increase the helical stability of two peptides from the activation domain of procarboxypeptidase A. Introduction of these same mutations in the protein results in a protein more resistant to urea or temperature denaturation.
15 December 1995*, Research Article, *Folding & Design*

► **Redesigning the substrate specificity of the hepatitis C virus NS3 protease**
Cristina Maria Failla, Elisabetta Pizzi, Raffaele De Francesco and Anna Tramontano. *Folding & Design* **1**, 35–42.

The hepatitis C virus (HCV) has a positive strand RNA genome of 9.5 kb, which contains a single open reading frame, encoding a polyprotein of ~3000 amino acids. The viral protein NS3 is a serine protease that bears homology to trypsin proteases and is responsible for proteolytic cleavage at some of the junctions in the polyprotein. The structure of NS3 is unknown. A model of the specificity pocket of NS3 was previously generated based on the known structures of trypsin-like proteases and on the conserved pattern of residues between different HCV strains. Here, the residues of the proposed specificity pocket are replaced by those from *Streptomyces griseus* protease B, changing the substrate specificity of the enzyme from Cys in the P1 position to Phe. This result confirms the prediction of the important catalytic residues in the model of NS3.
10 January 1996*, Research Article, *Folding & Design*

► **Crystal structure of the ternary complex of mouse lung carbonyl reductase at 1.8 Å resolution: the structural origin of coenzyme specificity in the short-chain dehydrogenase/reductase family**
Nobutada Tanaka, Takamasa Nonaka, Masayuki Nakanishi, Yoshihiro Deyashiki, Akira Hara and Yukio Mitsui (1996). *Structure* **4**, 33–45.

Mouse lung carbonyl reductase (MLCR) is a tetrameric oxidoreductase that catalyzes the NADPH-dependent reduction of a variety of carbonyl compounds, including xenobiotics, steroids and carbonyl compounds derived from lipid peroxidation. It is a member of the short-chain dehydrogenase/reductase (SDR) family, a group of enzymes that can be classified according to their preference (or requirement) for either NAD(H) or NADP(H). MLCR is the first protein in the SDR family to be crystallized with NADPH bound. Comparison with structures of other family members with NADH bound reveals that two basic residues are strongly indicative of a preference for NADPH (although neither one is sufficient on its own) and an aspartate residue near the cofactor binding site favors NADH binding. Thus, positively charged and negatively charged environments correlate with preference for NADPH and NADH, respectively. An (almost) conserved lysine side chain in MLCR has two functions, both stabilizing the position and orientation of the cofactor through a bifurcated hydrogen bond to hydroxyl groups of the ribose moiety, and stabilizing the position of the hydroxyl group of the tyrosine base catalyst while lowering its pK_a value.
15 January 1996, Research Article, *Structure*

► **The structure of the substrate-free form of MurB, an essential enzyme for the synthesis of bacterial cell walls**
Timothy E Benson, Christopher T Walsh and James M Hogle (1996). *Structure* **4**, 47–54.

Construction of the bacterial peptidoglycan layer requires cross-bridging by UDP-*N*-acetylmuramic acid, a compound that is synthesized by an enol ether transfer reaction, catalyzed by MurA, followed by the reduction of the enolpyruvyl moiety to a lactyl ether by MurB, which uses reduced nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. The structure of MurB in the absence of substrate is now compared with the previously determined substrate-bound structure, revealing the closure of the substrate-binding channel over the bound substrate. Two residues, Tyr190 and Lys217, hydrogen bond to the α-phosphate of the substrate and prevent passage of the substrate back through the channel that leads to and from the active site. In the substrate-free form of MurB, another residue, Tyr254, acts as a latch that holds Tyr190 away from the active-site cavity due to a stacking interaction.
15 January 1996, Research Article, *Structure*

► **The complex of the anti-cancer therapeutic, BW1843U89, with thymidylate synthase at 2.0 Å resolution: implications for a new mode of inhibition**
Thomas J Stout and Robert M Stroud (1996). *Structure* **4**, 67–77.

Thymidylate synthase (TS) catalyzes the rate-limiting step in the only biosynthetic pathway for thymidine. It is thus critical for DNA synthesis, and is an important target for anti-proliferative and anti-cancer drug design. The catalytic mechanism of TS involves the reductive methylation of dUMP by transfer of a methylene group from the cofactor methylenetetrahydrofolate ($C_2H_2H_4$folate), to produce deoxythymidine monophosphate (dTMP) and hydrofolate (H_2folate). The crystal structure of *E. coli* TS in a ternary complex with dUMP and BW1843U89, a folate analog, has been determined at 2.0 Å resolution. The binding of BW1843U89 to TS largely mimics that of the natural cofactor, however it causes a local deformation in the active site. The enhanced aromatic stacking between BW1843U89 and dUMP (compared to that of other cofactors and analogs for which the structure of the ternary complex is known) prevents Michael addition of Cys146-Sγ at C6 of dUMP, and may be a key component of the mechanism of inhibition by this analog. BW1843U89 is also unique among characterized inhibitors of TS in that it inactivates TS at a stoichiometry of one molecule to one TS dimer, which contains two, initially equivalent active sites. The binding of BW1843U89 to the first site changes the nature of the second active site, reducing the affinity of the second site for the inhibitor 1000-fold.
15 January 1996, Research Article, *Structure*

► **Solution structure of GroEL and its complex with rhodanese from small-angle neutron scattering**
P Thiyagarajan, SJ Henderson and A Joachimiak (1996). *Structure* **4**, 79–88.

Chaperonin 60 proteins are cylindrical oligomeric complexes that bind to unfolded proteins and assist in their folding. *E. coli* GroEL is a member of this family of proteins, and the crystal structure of GroEL has been previously determined. Here, the solution structure of this complex has been determined using small-angle neutron scattering (SANS) and shown to be similar to the crystal structure. The positions of the amino- and carboxy-terminal residues of each monomer subunit, which were disordered in the crystal structure, were determined and shown to be condensed near the equator of the cylinder. The SANS structure of GroEL in a complex with rhodanese shows that the unfolded protein is bound across the opening of the GroEL cavity, rather than within the complex as had been proposed. Instead of providing an infinite dilution environment by completely isolating the folding protein in its cavity, GroEL seems to provide a large active surface for the binding of unfolded proteins. The roles of the solvent and other cofactors thus have to be considered in determining the mechanism of chaperone-mediated protein folding.
15 January 1996, Research Article, *Structure*

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